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Lab Resource: Stem Cell Line

Genomic imprinting defect in *Zfp57* mutant iPS cell lines

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ABSTRACT

ZFP57 maintains genomic imprinting in mouse embryos and ES cells. To test its roles during iPS reprogramming, we derived iPS clones by utilizing retroviral infection to express reprogramming factors in mouse MEF cells. After analyzing four imprinted regions, we found that parentally derived DNA methylation imprint was largely maintained in the iPS clones with *Zfp57* but missing in those without maternal or zygotic *Zfp57*. Intriguingly, DNA methylation imprint was lost at the *Peg1* and *Peg3* but retained at the *Snrpn* and *Dlk1-Dio3* imprinted regions in the iPS clones without zygotic *Zfp57*. This finding will be pursued in future studies.

Resource table: *Zfp57* mutant iPS cell lines.

Name of stem cell construct	*Zfp57* knockout
Institution	Icahn School of Medicine at Mount Sinai
Person who created resource	Carol M. McDonald, Xiajun Li
Contact person and email	Xiajun Li, xiajun.li@mssm.edu
Date archived/stock date	October 18, 2011
Origin	Mouse MEF cells
Type of resource	Biological reagent: induced pluripotent stem cell (iPS)
Sub-type	cell line
Key transcription factors	Oct4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature (direct URL links and full references)	https://www.stemcell.ucla.edu/creating-ips-cells, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3727693/
Information in public databases	

Resource details

The properties of these isolated iPS clones

The iPS colonies started to form from the infected MEF cells grown on the SNL feeder cells in a week. We found that many iPS colonies also contained some cells that looked like transformed cells. We suspect that it may have been caused by retrovirus infection process or over-expression of reprogramming factors such as MYC during iPS cell derivation. We picked the colonies that displayed the best morphology with fewer "transformed cells" to establish iPS clones. The established iPS clones on feeder cells displayed a similar morphology to undifferentiated ES colonies, as exemplified by one *Zfp57*$^{+/-}$ (M^+Z^+) iPS clone, one *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clone and one *Zfp57*$^{-/-mz}$ (M^-Z^-) iPS clone (Fig. 1A). The genotypes for these iPS clones and parental MEF cells were confirmed by PCR-based genotyping (see Fig. 3B below). They formed embryoid bodies (EBs) when they were grown on non-adherent Petri dish plates (Fig. 1A). Based on semi-quantitative RT-PCR analysis, expression of the endoderm marker *Foxa2* seemed to be increased in EBs compared with iPS clones, in particular in two *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clones and one *Zfp57*$^{-/-mz}$ (M^-Z^-) iPS clone (Fig. 1C). By contrast, the mesoderm marker *Mlc2a* was expressed in both iPS clones and their EBs (Fig. 1C). We suspect that the expression of *Mlc2a* may reflect the parental origins of these iPS clones as they were derived from MEF cells. Interestingly, the ectoderm marker *Ck18* was highly expressed in the *Zfp57*$^{-/-mz}$ (M^-Z^-) iPS clone and its EBs although *Ck18* was not much expressed in one *Zfp57*$^{+/-}$ (M^+Z^+) and two *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clones but appeared to be modestly increased in the EB samples derived from these three iPS clones (Fig. 1C). To examine genome integrity of these iPS clones, we performed metaphase chromosome spread for four iPS clones, as exemplified by an image taken for one *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clone (Fig. 1B). Then we counted chromosome numbers and the results are summarized in Table 1. We did not find any euploid cells in one *Zfp57*$^{+/-}$ (M^+Z^+) clone (4.2–05) and one *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clone (4.3–01). By

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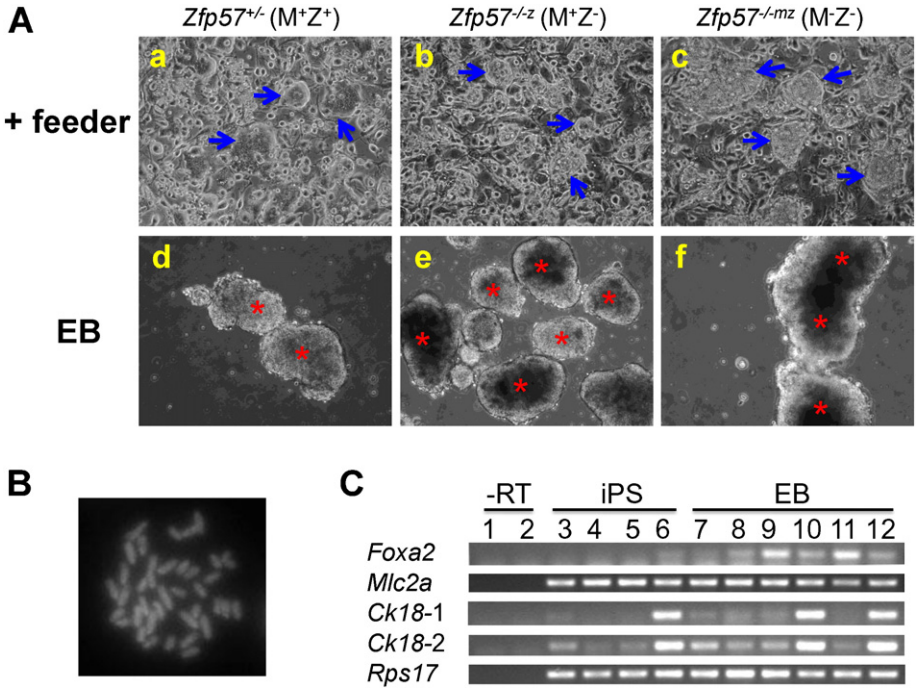

Fig. 1. Derived iPS clones display ES cell-like colonies on feeder cells and formed embryoid bodies (EBs) in suspension culture. A, the iPS clones were cultured on the SNL feeder cells (a, b, c) (McMahon and Bradley, 1990; Takahashi et al., 2007), or grown on non-adherent Petri dish plates for 7–8 days (d, e, f). One *Zfp57*$^{+/-}$ (M^+Z^+) iPS clone (a, d), one *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clone (b, e) and one *Zfp57*$^{-/-mz}$ (M^-Z^-) iPS clone (c, f) are shown here as the examples for the iPS clones derived from the MEF cells after retroviral infection-mediated expression of four reprogramming factors. Blue arrows in a-c, undifferentiated ES-like iPS colonies on top of the SNL feeder cells. Red asterisks in d–f, embryoid bodies (EBs). B, a DAPI-stained metaphase chromosome spread of one cell derived from a *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clone. C, semi-quantitative RT-PCR expression analysis of three marker genes in four iPS clones (lanes 3–6) and the EBs derived from these four iPS clones after growing in suspension culture for 8 days (lanes 7–10) or 10 days (lanes 11–12). Lanes 1–2, negative control without reverse transcription (−RT) of the same total RNA samples in lanes 4 and 5, respectively. Lane 3, one *Zfp57*$^{+/-}$ (M^+Z^+) iPS clone grown on the feeder cells. Lanes 4–5, two *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clones grown on the feeder cells. Lane 6, one *Zfp57*$^{-/-mz}$ (M^-Z^-) iPS clone grown on the feeder cells. Lane 7, day 8 EBs of the *Zfp57*$^{+/-}$ (M^+Z^+) iPS clone. Lanes 8–9, day 8 EBs of two *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clones. Lane 10, day 8 EBs of the *Zfp57*$^{-/-mz}$ (M^-Z^-) iPS clone. Lane 11, day 10 EBs of one *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clone. Lane 12, day 10 EBs of the *Zfp57*$^{-/-mz}$ (M^-Z^-) iPS clone. *Foxa2*, *Mlc2a* and *Ck18* are the lineage markers for endoderm, mesoderm and ectoderm, respectively. A house-keeping gene *Rps17* was used as the loading control here. 25 cycles of PCR amplification was used for *Rps17*, whereas 35 PCR cycles was applied to *Foxa2* and *Mlc2a*. For *Ck18*, 25 PCR cycles was performed first (*Ck18*–1), followed by additional five cycles of PCR amplification (*Ck18*–2).

contrast, roughly 20% euploid cells were observed in the other *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clone (4.3–04) and 35% of the cells in the *Zfp57*$^{-/-mz}$ (M^-Z^-) iPS clone (7.2–02) were euploid with 40 chromosomes (Table 1).

Expression of pluripotency markers

We analyzed expression of three pluripotency markers (OCT4, NANOG and SOX2) in one *Zfp57*$^{+/-}$ (M^+Z^+) clone (4.2–05), one *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clone (4.3–04) and the *Zfp57*$^{-/-mz}$ (M^-Z^-) iPS clone (7.2–02), together with the control wild-type ES cells (Fig. 2). We observed relatively high expression levels of OCT4 and SOX2 in all three iPS clones that were comparable to those of the wild-type ES cells. By contrast, we only observed high level of NANOG expression in the *Zfp57*$^{-/-mz}$ (M^-Z^-) iPS clone but not in the *Zfp57*$^{-/-z}$ (M^+Z^-) iPS clone. There were a few strong NANOG-positive cells present in the *Zfp57*$^{+/-}$ (M^+Z^+) clone. Since OCT4 and SOX2 are two reprogramming factors used for the derivation of these iPS clones, expression of OCT4 and SOX2 could be either activated from the endogenous loci after reprogramming or expressed from the integrated retroviruses carrying the *Oct4* and *Sox2* transgenes. Further research is needed to distinguish these possibilities.

DNA methylation imprint in iPS clones

Genomic DNA samples were harvested from the control wild-type ES cell, *Zfp57* mutant tail sample, parental MEF cells and derived iPS clones. Their genotypes were confirmed by PCR-based genotyping (Fig. 3B). Due to the presence of trace amount of feeder cells and preferential amplification of the shorter PCR amplicon, a small portion of the PCR product was amplified from the wild-type allele of *Zfp57* in the genomic DNA samples of three *Zfp57*$^{-/-}$ mutant iPS clones (see lanes 11–13 of Fig. 3B). COBRA analysis was performed for these genomic DNA samples. We analyzed DNA methylation imprint at the *Snrpn*, *Peg1*, *Peg3* and *Dlk1-Dio3* imprinted regions (Fig. 3A). Previously, we found that ZFP57 maintains DNA methylation imprint at these four imprinted regions in mouse embryos and ES cells (Li et al., 2008; Zuo et al., 2012). As expected, both methylated and unmethylated DNA products were present at these four imprinted regions in the wild-type ES cells after COBRA (lane 1 of Fig. 3A), whereas only unmethylated DNA was

Table 1
Counting of metaphase chromosome spreads of four iPS clones.

iPS clone	4.2–05	4.3–01	4.3–04	7.2–02
Genotype	*Zfp57*$^{+/-}$ (M^+Z^+)	*Zfp57*$^{-/-z}$ (M^+Z^-)	*Zfp57*$^{-/-z}$ (M^+Z^-)	*Zfp57*$^{-/-mz}$ (M^-Z^-)
# of counted metaphase spreads	20	9	20	20
# of spreads with 40 chromosomes	0	0	4	7
% of euploid cells	0	0	20	35